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Geometrical considerations in cardiac electrophysiology and arrhythmogenesis

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Abstract

The rate of repolarization (R_{Repol}) and so the duration of the cardiac action potential is determined by the balance of inward and outward currents across the cardiac membrane (net ionic current). Plotting action potential duration (APD) as a function of the R_{Repol} reveals an inverse non-linear relationship, arising from the geometric association between these two factors. From the R_{Repol}-APD relationship it can be observed that a longer action potential will exhibit a greater propensity to shorten, or prolong, for a given change in the R_{Repol} (i.e. net ionic current), when compared to one that is initially shorter. This observation has recently been used to explain why so many interventions that prolong the action potential exert a greater effect at slow rates (reverse rate-dependence). In this article we will discuss the broader implications of this simple principle and examine how common experimental observations on the electrical behavior of the myocardium may be explained in terms of the R_{Repol}-APD relationship. An argument is made, with supporting published evidence, that the non-linear relationship between the R_{Repol} and APD is a fundamental, and largely overlooked, property of the myocardium. The R_{Repol}-APD relationship appears to explain why interventions and disease with seemingly disparate mechanisms of action have similar electrophysiological consequences. Furthermore, the R_{Repol}-APD relationship predicts that prolongation of the action potential, by slowing repolarization, will promote conditions of dynamic electrical instability, exacerbating several electrophysiological phenomena associated with arrhythmogenesis, namely, the rate-dependence of dispersion of repolarization, APD restitution and electrical alternans.

Keywords: action potential duration; reverse rate-dependence; dispersion of repolarization; restitution; alternans; repolarization dynamics.

Article

In 2006 Zaza and Varró published a novel hypothesis on the mechanisms by which class III anti-arrhythmic drugs act to act to prolong the duration of the cardiac action potential to a greater extent at slow rates (so called reverse rate-dependence (RRD)).[1] Their principle observation, which is discussed in more detail later in this article, was that RRD is an intrinsic property of the myocardium; a consequence of the non-linear relationship between action potential duration (APD) and the rate of repolarization (R_{Repol}) (or net ionic current across the cardiac membrane, see Figure 1). This idea, which follows from observations on the importance of membrane potential velocity in governing intrinsic heart rate variability,[2, 3] has recently been validated in experimental studies.[4, 5] The purpose of the present article is to highlight the broader importance of this simple principle. In this article we will examine how common experimental observations, on the electrical properties of the myocardium, may be understood in terms of the non-linear R_{Repol}-APD relationship. This simple principle appears to explain why many factors that modify APD, but by disparate modes of action, have similar electrophysiological consequences. We will argue, using existing published data, that the non-linear relationship between R_{Repol} and APD is a fundamental, and largely overlooked, property of the myocardium. The R_{Repol}-APD relationship predicts that prolongation of the action potential, by slowing repolarization, will promote conditions of dynamic electrical instability, exacerbating several rate-dependent phenomena associated with arrhythmogenesis (i.e. steeper dependence of regional repolarization gradients on heart rate, augmented APD restitution (APD-RT) kinetics and the amplification of electrical alternans). These ideas and their potential clinical implications will be discussed.

The rate of repolarization-action potential duration relationship

In order to understand the observations of Zaza and Varró,[1] and experimental validation of Nánási and colleagues,[4, 5] we must first consider the following equation, which tells us that the total membrane current (I_{Total}) can be calculated from the product of the capacitive and ionic currents.

$$I_{Total} = C_m \frac{dV}{dt} + I_{Ionic}$$

(Equation 1) [4, 6]

where C_m represents the membrane capacitance, dV/dt the change in transmembrane voltage over time and I_{Ionic} the total ionic current. If all transmembrane elements are subject to the same membrane potential then total membrane current (ionic + capacitive) must necessarily equal zero (see Zaza[7]). Therefore, we can simplify to Equation 2.

$$I_{Ionic} = -C_m \frac{dV}{dt}$$

(Equation 2) [4, 6]

This says that the ionic current is equal to the product of membrane capacitance and the rate of change of transmembrane potential (with an opposite sign). Thus, we can estimate net ionic current from the first derivative of the membrane potential waveform. This is the methodology used by Bányász *et al*. and Bárándi *et al.* who plotted APD as a function of the net ionic current at 50% repolarization to generate Figures 1A & 1B, respectively.[4, 5] We can also observe that, as the membrane capacitance is fixed, any change in net ionic current (i.e. the balance of inward and outward currents) will generate a proportional change in the R_{Repol}. In this regard, if we assume that the R_{Repol} is constant, Figure 1 can be seen to arise as a result of the geometrical relationship between the slope of repolarization and the duration of the action potential (see Figure 2). Moreover, as the same relationship can be derived from experimental action potential recordings, when APD is plotted a function of the average R_{Repol} during repolarization (see Figure 1C&D), this is not simply an artifact of selecting a single arbitrary point during repolarization. Complications arising from a notch and dome shaped action potential to the relationship between APD and R_{Repol} have been discussed previously.[4]

The upshot of all this is that a longer action potential will exhibit a greater propensity to shorten, or prolong, for the same magnitude change in the R_{Repol} (i.e. change in I_{Ionic}), simply because the initial R_{Repol} is already less steep. This is the principle reason why class III anti-arrhythmic drugs act in a reverse rate-dependent manner (see below). Note that alterations in the R_{Repol} during the plateau, when dV/dt is small, will have the greater impact on APD than those that occur later in repolarization. There is also some evidence that the magnitude of the inwardly rectifying (I_{K1}) and rapid delayed rectifying (I_{Kr}) potassium currents are related to the shape of the action potential, being potentiated at faster R_{Repol}.[8, 9] Such "self-augmentation" would increase the magnitude of change in APD, whereby an slowing of the R_{Repol} during the plateau would also slow terminal repolarization, however, not all studies support this hypothesis[10].

In the remainder of this article we will discuss the findings, and implications, of recent studies on the RRD of class III anti-arrhythmic drugs and the role of the R_{Repol}-APD relationship as an underpinning for common experimental observations in cardiac electrophysiology. Discussion will primarily focus on alterations in rate-dependent phenomena at the level of the ventricles in larger mammalian species (i.e. guinea pig, rabbit, dog and man).

Reverse rate-dependence of anti-arrhythmic drugs

The maintenance of re-entrant arrhythmia depends upon conduction velocity and refractory period, the so-called cardiac wavelength (i.e. the product of conduction velocity and the functional refractory period). Factors that decrease conduction velocity, or shortening refractory

period, may facilitate arrhythmia because they will reduce the minimum volume of tissue required for functional re-entry.[11] Class III anti-arrhythmic agents block outward potassium current to prolong repolarization, and the refractory period, and so may prevent re-entry by increasing the cardiac wavelength. An ideal class III agent is one that exhibits a positive rate-dependence, prolonging APD to a greater degree at fast rates, such as during ventricular tachyarrhythmia. However, the development of class III anti-arrhythmic drugs with positive rate dependence has proved elusive.[4, 12] Drugs that reduce outward potassium current are commonly reported to prolong repolarization to a greater degree at slow heart rates. [4, 12] As such, class III anti-arrhythmic drugs would be predicted to be less effective during tachyarrhythmia. Furthermore, augmented action potential prolongation with bradycardia may trigger early afterdepolarisations and torsade de pointes (TdP), a polymorphic ventricular tachycardia associated with syncope and sudden death. The failure of pure class III anti-arrhythmic agents to prevent death from ventricular arrhythmia in clinical trials[13, 14] and conflicting results of experimental studies (see Table 6 in Tsuchihashi & Curtis[15]) may be explained by this lack of efficacy at faster rates and the adverse consequences of RRD.

Although several mechanisms have been proposed to underpin the RRD of class III anti-arrhythmic agents, including the mode of drug-channel interactions,[16] ion channel kinetic properties,[17] and potassium accumulation in the sarcolemmal cleft,[18] recent evidence has highlighted the non-linear relationship between net ionic current (i.e. the R_{Repol}) and APD as an important contributory mechanism. Bányász *et al*. investigated the effect of pharmacological interventions, and the direct injection of current, on the rate-dependence of APD in isolated canine ventricular myocytes.[4] Data from this study are reproduced in Figure 3, the principle finding of which is that there exists a commonality between interventions that modulate APD and their impact on APD rate-dependence. Despite different mechanisms of action, pharmacological interventions that prolong APD were found act in a RRD manner. This includes $BaCl_2$ (an inhibitor of I_{K1}), dofetilide (an inhibitor of I_{Kr}), Bay K8644 (an agonist of the L-type inward calcium current, I_{Cal}), and veratradine (an agonist of the inward sodium current, I_{Na}) Similarly, interventions that shorten APD (namely, nicrorandil and lidocaine) were found to have a greater effect at slow rates (see Figure 3A). Importantly, Bányász *et al.* went on to demonstrate that pharmacological RRD could be mimicked by the injection of outward and inward currents during the action potential (Figure 3B). This indicates that RRD is unlikely to be due to specific ion channel kinetics or mechanisms of drug action, but arises primarily as an intrinsic property of the cardiac membrane. In support of this, injection of inward current was shown to abolish the RRD prolongation of APD with $BaCl_2$ inhibition of I_{K1} (Figure 3C). The authors concluded that the non-linear relationship between I_{Ionic} and APD is an important principle underpinning RRD, though they note that this does not exclude a role for other mechanisms (e.g. the nature of drug-channel interactions). Importantly, in a follow-up study, it

was shown that the magnitude of change in APD with various pharmacological interventions is not a direct function of heart rate but is actually determined by the initial APD.[5] Slowing heart rate prolongs APD and so the magnitude of response (to a drug) is observed to be greater at slow rates. From our earlier discussion we can see why this is the case. Assuming that a given intervention has the same net effect at all heart rates we can observe that a longer action potential will exhibit a greater propensity to shorten, or prolong, for the same change in R_{Repol} (i.e. I_{Ionic}), in comparison to an action potential of shorter initial duration. This is the geometric relationship illustrated in Figure 2. The implication here is that developing selective class III anti-arrhythmic agents with an absence of RRD is unlikely to be successful.

Rate dependency of dispersion of repolarization

It is well established that ventricular APD, and repolarization times, are spatially and temporally inhomogeneous[19-21] and there is convincing experimental evidence to indicate that dispersion of repolarization is influenced by heart rate. The vast proportion of experimental data, primarily from studies in the dog, indicates that slowing of rate, by pacing or autonomic nerve stimulation, acts to magnify regional differences in APD.[22-34] This observation may be best explained by the R_{Repol}-APD relationship.

In the preceding section we discussed how the initial duration of the action potential (i.e. R_{Repol}) determines the magnitude of prolongation when heart rate is slowed (in isolated ventricular myocytes). The corollary of this is that in the intact heart bradycardia would be expected to magnify regional differences in APD. If we assume that a slowing of rate results in a uniform decrease in the R_{Repol} throughout the ventricles then a greater prolongation of APD would be expected to occur in regions where APD is relatively prolonged, in comparison to those that APD is relatively short, due to the non-linear R_{Repol}-APD relationship. This can be fully appreciated from Figure 4A, which illustrates the predicted effect of slowing heart rate (modeled as a uniform slowing in the R_{Repol}) on the APD dispersion. The initial difference in APD between two regions (R1 and R2) increases when heart rate is slowed. Furthermore, the magnitude of change in dispersion with bradycardia will directly depend upon degree of APD heterogeneity at baseline. Any factor (drug or disease) that increases the heterogeneity of APD, in the absence of some opposing effect, would be predicted to exacerbate the increase in APD dispersion on the slowing of heart rate. This can be readily appreciated from Figure 4B and existing experimental evidence supports this hypothesis. For example, we recently showed that bradycardia, induced by electrical stimulation of the vagus nerve, increases apicobasal dispersion of repolarization in the left ventricle of isolated perfused rabbit hearts (see Figure 4C).[35] Treatment with E4031, a selective blocker of I_{Kr} conductance, prolongs APD, increases region-to-region differences baseline APD and augments the rate-dependent increase in dispersion associated with vagus nerve stimulation. This is illustrated by the linear correlation between the initial APD and the magnitude of APD prolongation (Figure 4D), where bradycardia

acts to prolong the action potential to a greater extent in regions with longer baseline APD. A similar dependence on initial conditions has been reported in dogs with pharmacologically induced long QT type 3 (I_{Na} dependent), whereby increased APD heterogeneity at baseline is associated with a potentiation of the increase in dispersion observed when heart rate is slowed.[23, 33] Augmented rate-dependence of dispersion of repolarization has also been reported in mice with congenital long QT type 3.[31] As such, the relationship between greater regional APD heterogeneity, and its augmentation at slow rates, does not appear to be specifically related to one class of ion channels, or drugs, and may be generally understood in terms of the APD-R_{Repol} relationship.

From a clinical perspective bradycardia is known to be predisposing factor for the occurrence of the TdP in pharmacologically acquired long QT syndrome[36] and death commonly occurs at night in congenital long QT type 3, when vagal tone is high and heart rate relatively slowed.[37] Experimental models of acquired (pharmacological inhibition of I_{Kr}) and congenital type 3 long QT syndrome indicate that these conditions are associated with increased spatiotemporal dispersion of repolarization and that this is exacerbated at the slow rates required to trigger TdP.[28, 29, 31, 32] Non-uniformities of APD, secondary to bradycardia, can provide a substrate for functional conduction block and re-entrant excitation, with early afterdepolarisations acting as a focal trigger.[33] Both focal and re-entrant mechanisms are thought to contribute to the initiation and maintenance TdP in long QT syndrome.[33, 38]

Action potential duration restitution

In most larger mammalian species (i.e. human, dog, guinea-pig) a decrease in the inter-beat coupling interval or, more correctly, diastolic interval (DI) results in a shortening of the ventricular action potential. The APD-RT curve, as illustrated in Figure 5, plots APD as a function of a decreasing DI and approximates a mono-exponential curve (the bi-phasic rabbit APD-RT curve, demonstrating APD prolongation (with decreasing DI) at long cycle lengths, is a notable exception – although the rabbit is largely mono-phasic over physiological heart rates[39]). APD-RT involves a number of ionic processes, including, a reduction of intracellular calcium transient amplitude leading to a decrease of inward current through the sodium-calcium exchanger,[40] incomplete reactivation and a decrease of the inward calcium current,[40, 41] incomplete deactivation and an increase of outward K^+ currents[40, 42] and self-augmentation of outward K^+ currents.[8] The net effect is that as DI is decreased there is a reduction of inward and increase in outward current, resulting in a shorter action potential. This capacity of the action potential to adjust to a sudden perturbation in rate clearly plays a important physiological role; accommodating fast heart rates, reducing the risk of conduction block when rate suddenly increases (fight or flight) and, conversely, preventing early re-excitation when rate is slowed. However, the APD-RT curve is also implicated in ventricular arrhythmogenesis. The slope of the restitution curve has been proposed as a mechanism whereby organized ventricular tachycardia

can transition to “chaotic” ventricular fibrillation.[43] APD-RT is also implicated in the mechanisms mediating re-entrant arrhythmia, whereby regional restitution properties act, in concert with the timing of a premature stimulus, to determine the occurrence functional re-entry[44] This is similar to computational simulations suggesting that spatial heterogeneity of APD-RT is a pro-arrhythmogenic substrate.[45]

As the APD-RT curve depends upon a number of cellular processes it is reasonable to assume that factors that alter the duration of the action potential will have different effects depending upon their specific mechanism of action. However, a review of the literature reveals a startlingly consistent relationship between modulation of APD and its impact on the APD-RT curve. Factors that act to prolong APD are commonly reported to increase the kinetics of the APD-RT relationship, demonstrating a greater shortening of APD over the same range of DIs, and an increase in APD-RT slope. These factors include bradycardia,[46] experimental heart failure,[47] excitation-contraction uncoupling agents[48, 49] and the action of drugs that prolong APD by reducing outward K^+ current[50-52] or increasing inward Na^+ current.[53] By comparison, factors that shorten APD act to flatten the APD-RT curve (see Figure 5).[50, 54-56] It is, however, not immediately apparent why interventions with seemingly disparate mechanisms of action have similar electrophysiological consequences. This observation cannot simply be ascribed to changes in intracellular ion accumulation, as this differs in each case. For instance, whilst bradycardia causes a reduction in both intracellular sodium ($[Na^+]_i$) and calcium ($[Ca^{2+}]i$), potassium channel block will increase $[Ca^{2+}]_I$ without effect on $[Na^+]_i$ and sodium channel activation will elevate both $[Na^+]_i$ and $[Ca^{2+}]_I$. Despite this, all 3 interventions have been shown to increase the magnitude of APD-RT.[46, 50-53] Additionally, it is difficult to understand why interventions that would be predicted to flatten the APD-RT curve (e.g. a decrease in K^+ conductance) actually have the opposite effect.[50-52] We propose that this disparity may be understood by considering the non-linear relationship between R_{Repol} and APD.

Figure 5A illustrates two theoretical APD-RT curves generated using the R_{Repol}-APD relationship. Starting from a predefined APD (circles=normal, squares=prolonged) the process of electrical restitution is modeled as a series of incremental step increases in the $R_{Repol,}$, with estimated APD plotted, at each step, against a predefined range of DIs. Note that the range of DIs here is essentially arbitrary and is the same in both curve. As such, Figure 5A models the increase in net outward current with decreasing DI and allows us to predict the effect of altering the initial duration of the action potential on the APD-RT curve. From Figure 5A we can see that restitution of APD is predicted to be of greater magnitude, and the slope of the APD-RT curve steeper, when initial APD is longer. By definition this will arise independently of other factors that may influence restitution (e.g. changes in intracellular calcium load or sodium channel gating). Moreover, in accordance with studies on AP adaptation,[4, 5] this is expected to be somewhat independent of the mechanism by which APD is prolonged (i.e. specific ionic

conductance(s)). To reiterate this point, the implication from the R_{Repol}-APD relationship is that magnitude of APD-RT is, in part, a function of the initial APD and that this explains the commonality that factors that prolong APD also increase the kinetics of the APD-RT curve. Note that, in Figure 3A, due to the exponential nature of the R_{Repol}-APD relationship, the two APD-RT curves converge at the same point. This observation of converging curves is seemingly a universal feature of experimental studies in which APD-RT is compared before and after an intervention that modifies APD (with recordings made either in the same cell / region of the heart or in matched samples) and several examples of this pattern are presented in Figure 5. The fact that this is so consistently observed suggests a common mechanism and this is likely to be the R_{Repol}-APD relationship.

It is clear from Figure 5 that, while APD prolongation generally increases the kinetics of restitution process, there are some notable differences in the profile of the APD-RT curve between interventions. This serves to highlight the fact that whilst the R_{Repol}-APD relationship may play an important role it is not the only factor that will influence APD-RT. If, for example, a particular ion channel, or pump, contributes to the shortening of APD at very short DIs (e.g. I_{Cal}) then this would clearly impact on the shape of the terminal portion of the APD-RT curve. Similarly, if an intervention modifies intracellular calcium handling this may alter the calcium dependent portions of the curve, reducing or augmenting the change in ionic current over a given range of DIs.

Electrical alternans

Electromechnical cardiac alternans refers to the cyclic variation in the duration of ventricular APD (long-short-long-short) and intracellular calcium transient (large-small-large-small) observed at rapid heart rates.[57] Clinically this takes the form of macro or microscopic oscillations in the amplitude and/or duration of the electrocardiographic T-wave. Alternans are commonly observed in conditions associated with increased risk of arrhythmia[56, 58-62] and clinical trials have established that microvolt T-wave alternans are a predictor of risk of tachyarrhythmia and sudden cardiac death.[63, 64] It is beyond the scope of this article to discuss the multitude of cellular and multicellular properties that contribute to the phenomenon of cardiac alternans and readers requiring additional information are directed to Weiss et al.[57] We will instead focus on the impact of a change in APD on cardiac alternans and the proposed of the importance of the R_{Repol}-APD relationship.

Existing experimental evidence indicates that the magnitude of electrical alternans is increased by interventions that prolong[49, 65-67], and decreased by those that shorten,[49, 67-69] the action potential. Figure 6A, with data from Fossa et al.,[60] demonstrates the occurrence of significant APD alternans with rapid pacing in guinea pig hearts, in the presence of E4031 but not in control conditions. As with APD-RT, the potentiation of alternans appears to be a function of the duration of the action potential rather than a property related to any individual ion channel. In

support of this we can observe that alternans are potentiated by interventions that prolong APD by increasing inward Na^+ current [70, 71] or decreasing outward K^+ currents.[65] Moreover, data from Fujimoto et al. demonstrates that the increase in alternan amplitude with disopyramide mediated APD prolongation can be reversed by nicorandil, an activator of the ATP sensitive potassium current.[67]

Altering the duration of the action potential will influence cardiac alternans through several interrelated mechanisms. The first is that when the action potential is prolonged there will be an increase Ca^{2+} entry through $I_{Cal,}$ which will enhance $[Ca^{2+}]_I$ and amplify beat-to-beat oscillations in calcium transient amplitude (and so APD). Secondly, the R_{Repol}-APD relationship predicts that prolonging repolarization will magnify the beat-to-beat oscillation in APD associated with cycling of intracellular calcium (Figure 6A). The amplitude of the calcium transient directly modulates the action potential by potentiating inward current through the sodium-calcium exchanger (NCX) and / or reducing inward current through I_{Cal} but may also activate calcium-dependent chloride currents, [72] I_{K1},[73] I_{to}[74] and the delayed rectifying K^+ currents.[75] The relative balance of effects on calcium-dependent ionic conductances may result in positive and negative calcium-voltage coupling phenomena, though the former, where an increase in calcium transient amplitude prolongs the action potential, is more commonly observed.[57] In either case the calcium transient will influence the R_{Repol} and, as we have already discussed, a longer action potential will demonstrate a greater propensity to shorten, or prolong further, for a fixed change in the R_{Repol}. Therefore, any factor that prolongs APD would be predicted to increase the amplitude of electrical alternans. This idea is illustrated in Figure 6C, where a beat-to-beat oscillation in the calcium transient drives a beat-to-beat oscillation in the R_{Repol}. When APD is prolonged the same magnitude oscillation in the R_{Repol} gives rise to APD alternans of greater amplitude. It will be immediately obvious that this observation relates back to our previous discussion on APD-RT and it is already well established that a steeper restitution slope enhances the magnitude of electrical alternans.[57] A final related factor is that for a given heart rate (i.e. fixed cycle length) the DI between subsequent beats will, by definition, be less when the action potential is prolonged. As such, hearts will operate on the steeper portion of the APD-RT curve, enhancing beat-to-beat alternation of APD (see Figure 6A&B).

In the setting of spatially discordant alternans, in which regional APD oscillations are out of phase, the R_{Repol}-APD curve predicts that having a longer action potential to begin with will exacerbate spatial heterogeneities of repolarization, even if APD is uniform across the ventricles at baseline. This can be observed Figure 6A, where the difference between long and short beats during concordant alternans also reflects spatially gradients of repolarization between two regions during discordant alternans. This is important because it is discordant alternans that have been shown to be pro-arrhythmogenic. Discordant alternans may increase the likelihood that an ectopic stimulus will trigger re-entry, and may also promote spontaneous

tachyarrhythmia, by generating a suitable substrate for arrhythmia (i.e. increased spatiotemporal dispersion of repolarization).[57] APD prolongation will exacerbate this and so may increase susceptibility to arrhythmia.

Clinical and technical implications

Abnormal prolongation of the electrocardiogram QT interval, a measure of the ventricular APD, is associated with increased risk of death from malignant ventricular arrhythmia. Inherited channelopathies and drug induced long QT syndrome represent the most obvious examples, however there is also substantial evidence linking QT prolongation to ventricular arrhythmogenesis, and premature mortality, in coronary artery disease,[76] chronic heart failure[77], alcoholic liver disease,[78] and in some studies of hypertrophic cardiomyopathy.[79] Most strikingly, several large-scale population studies indicate that QT prolongation predicts cardiovascular mortality in the general population.[80-82] In recent decades there has been substantial progress towards understanding the cellular mechanisms linking prolonged repolarization to triggered electrical activity (early afterdepolarisations) and TdP.[83] The principles discussed in this article suggest that QT prolongation will also promote dynamic electrical instability, because a proportional change in the R_{Repol} results in a greater magnitude change in the APD when repolarization is already slowed / prolonged. As such, it is hypothesized that abnormal prolongation of the action potential, in any condition, may exacerbate electrophysiological phenomena that are associated with malignant ventricular arrhythmia, including, dispersion of repolarization, APD-RT kinetics and the magnitude of electrical alternans. Furthermore, the R_{Repol}-APD relationship predicts that prolonging the action potential will facilitate instability whislt exerting little effect on the cardiac wavelength during tachyarrhythmia.[4] These observations may be of some importance in understanding of the conflicting results of animal studies using class III anti-arrhythmic agents[15] and the failure of pure class III drugs in the clinical management of ventricular arrhythmia.[13, 14]

From a technical standpoint, the fact that a uniform slowing of repolarization will act to magnify intrinsic differences in APD (see Figure 4) makes it impossible to attribute a heterogeneous change in regional repolarization to non-uniformities in ion channel expression, a fairly common assumption in experimental studies. To correctly establish whether the effect of an intervention on regional APD can be attributed to regional ion channel expression investigators must also consider the initial APD. Assessing the proportional change in the average R_{Repol}, as well as APD, may prove to be more informative. There are additional issues of interpretation that arise when investigating an intervention that modifies APD. For instance, if we were to take a myoycte and measure the APD-RT curve before and after drug treatment we might observe, as discussed, a prolongation of APD and a steepening of the restitution relationship. Thus we might conclude that the ion channel we have targeted has a role in restitution. In some respects this is true, however, it does not tell us that the ion channel in question directly contributes to the

adaptation of APD during restitution. As we have already observed, prolongation of APD would be expected to influence the APD-RT curve indirectly. Thus, in order to establish the importance of a particular ionic conductance it is essential to control for the difference in APD between groups. Of course this may be regarded as a somewhat redundant point as the change in APD is a direct consequence of the intervention on its target ion channel or pump and so its electrophysiological action.

Limitations

In this article we have discussed the importance of the R_{Repol}-APD relationship as an underpinning for common electrophysiological observations, whereby a change in the duration of the action potential has predictable consequences that appear to be somewhat independent of the mechanism by which APD is modified. It is not, however, our intention to suggest that this is the only factor that warrants consideration. Interventions that modify ion channel behavior and / or intracellular ionic composition may effect both the duration of the action potential but also the response of the membrane to a given stimulus (e.g. a premature beat). Such factors may act in concert or in opposition. Furthermore, ion channel modulating drugs can demonstrate either use- or reverse use-dependent block, binding to their target ion channel in the open, inactive or resting state. As such, the degree of channel block can vary directly, or inversely, with heart rate and may vary from drug to drug. Such drug-channel interactions may oppose or augment the RRD attributed to the action potential morphology. The R_{Repol}-APD relationship provides a means to predict the effect of any intervention that alters APD, however, on its own it may be insufficient to fully explain the electrophysiological changes with drugs or disease.

It is well established that the cardiac ion channels make a varying contribution at different stages of repolarization, owing to their voltage, time and ionic dependencies. As such, the approach of measuring repolarization at a single defined point during repolarization could be criticized. A change in the R_{Repol} occurring late in repolarization would be expected to have less of an impact on the APD then a change that occurs earlier, however this could be addressed by using the average R_{Repol}, as in Figure 1D.

Summary

In this article an argument has been made that the non-linear R_{Repol}-APD relationship explains why interventions and disease with disparate modes of action have similar electrophysiological consequences. The simple fact that a longer action potential will demonstrate a greater propensity to shorten or prolong, for a given change in the R_{Repol}, has important implications. With the exception of studies addressing the mechanisms of RRD it appears that this principle has been largely overlooked. The R_{Repol}-APD relationship predicts that QT prolongation will generate conditions of dynamic electrical instability, exacerbating several electrophysiological phenomena associated with ventricular arrhythmia.

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Figure legends

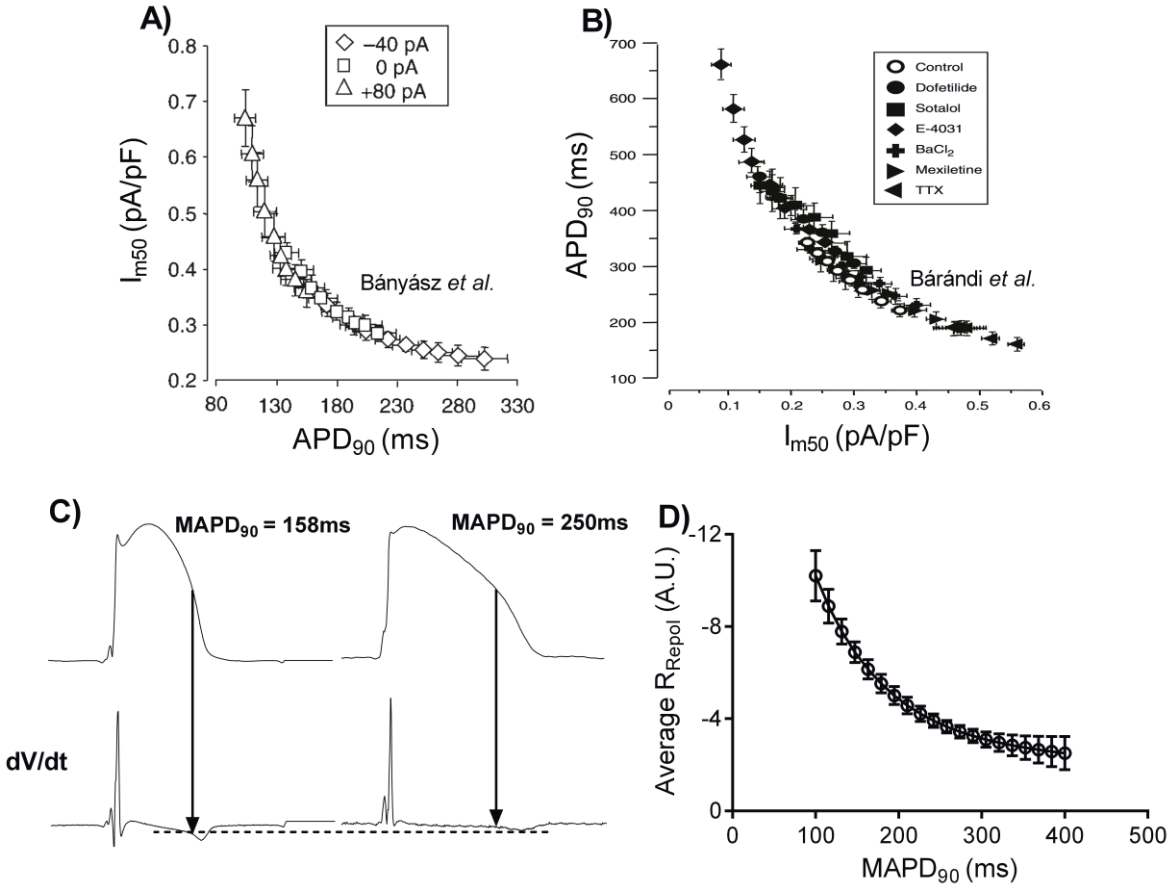

Figure 1. Action potential duration is a non-linear function of the rate of repolarization (net ionic current). A&B) Data from isolated ventricular myocytes demonstrating the non-linear relation between action potential duration (APD) and net ionic current at 50% repolarization (I_{m50}). APD was modified by A) direct current injection (outward – diamonds, inward – triangles) and B) by pharmacological interventions. Reproduced with permissions from Bányász *et al*.[4] and Bárándi *et al.*[5], respectively. C) Example monophasic action potential recordings, along with their first derivatives, showing the relationship between monophasic APD (MAPD) and the rate of repolarization in an isolated rabbit heart. Arrows denote 50% repolarization (unpublished observations). D) Plotting monophasic action potential duration ($MAPD_{90}$) as a function of the average rate of repolarization reveals a non-linear relationship comparable to that observed in isolated myocytes. MAP recordings were made over range of heart rates in rabbit hearts in control conditions and in the presence of E4031 (50nM), a selective inhibitor of the rapid delayed rectifying potassium current. For analysis the amplitude of the plateau was normalized to 1. Average repolarization was assessed as the mean of the first derivative of the action potential waveform from the peak of the plateau (i.e. after the notch) to 90% repolarization (n=5, unpublished observations).

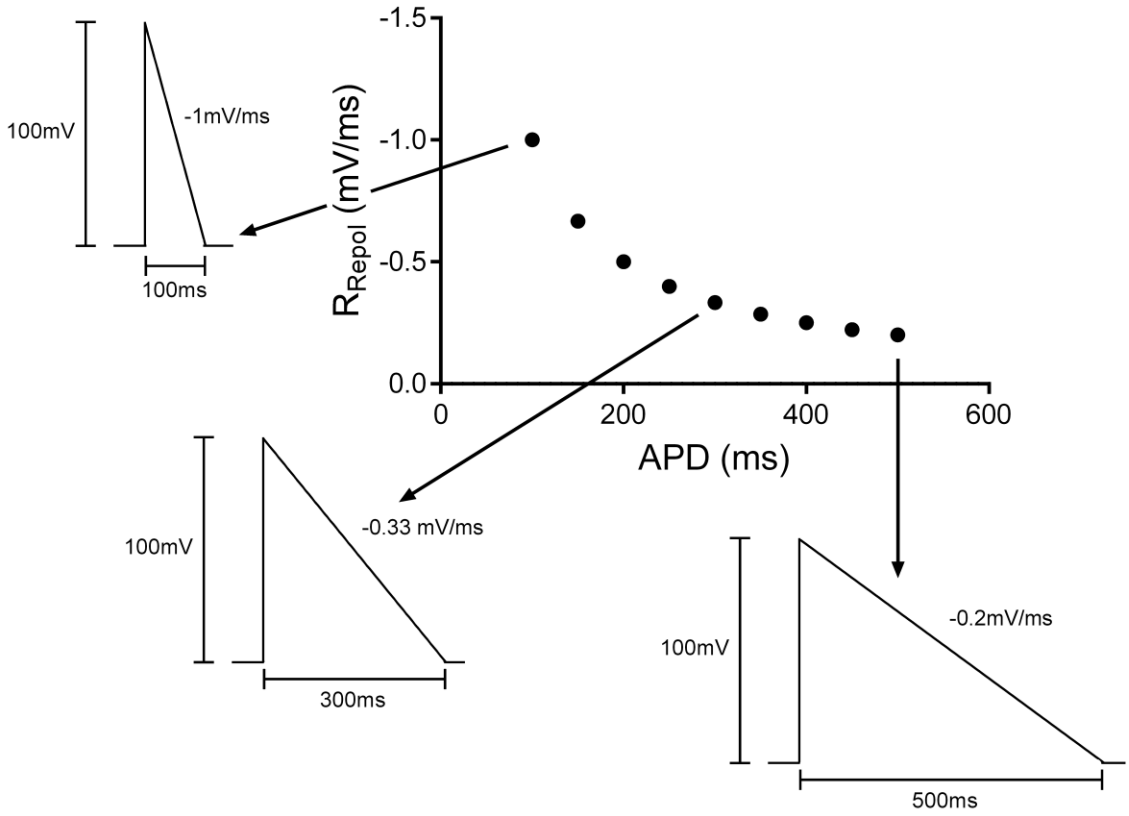

Figure 2. An intrinsic geometrical relationship between the rate of repolarization and action potential duration. Representative diagram. R_{Repol}=Rate of repolarization, APD=action potential duration.

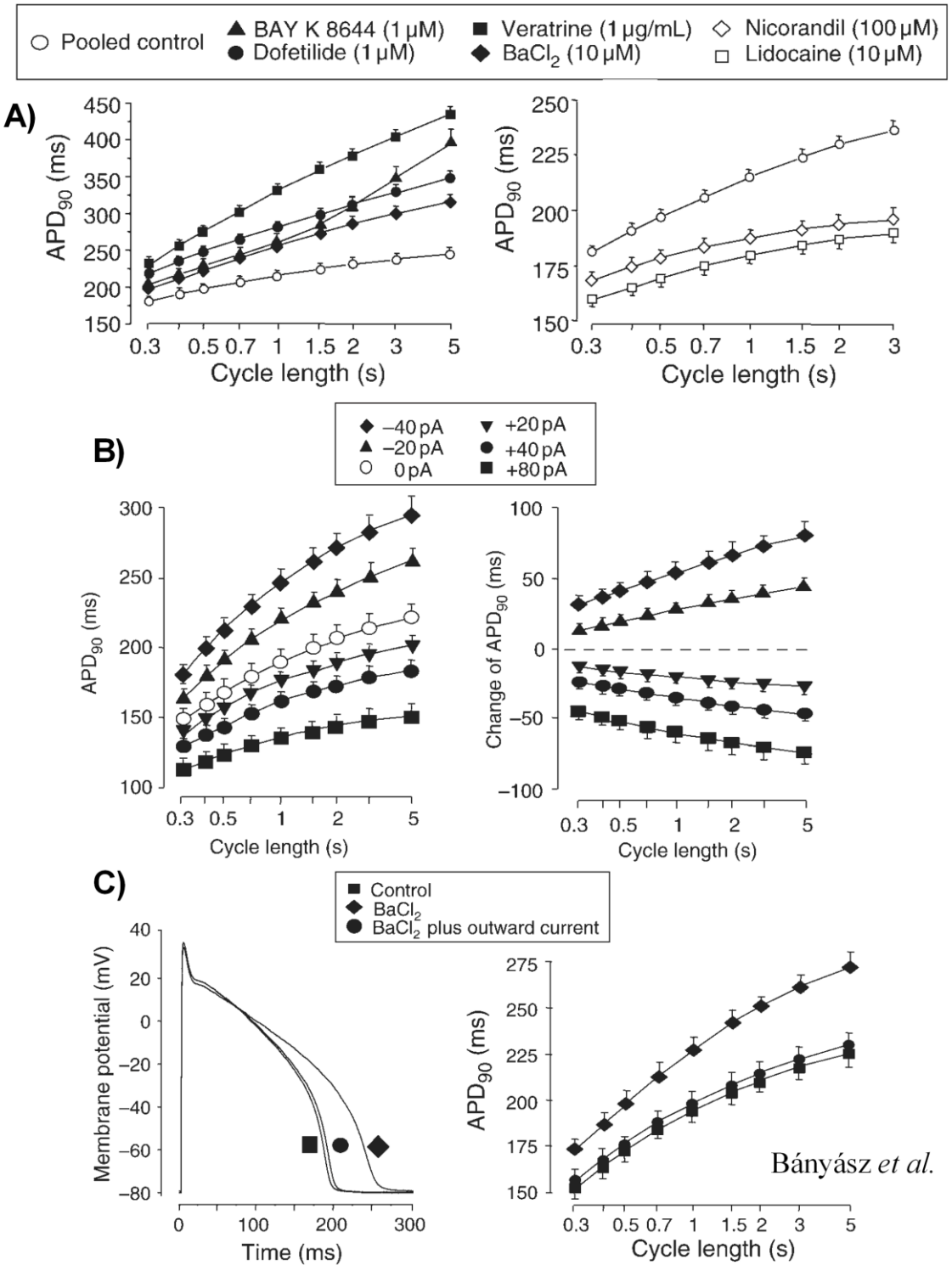

Figure 3. Reverse rate-dependence is an intrinsic property of the cardiac membrane. Data from isolated canine ventricular myocytes demonstrating the reverse rate-dependent modulation of action potential duration (APD) with A) drugs that prolong and shorten repolarization and B) direct injection of inward (+) and outward (-) current during microelectrode recordings. C) Reverse rate-dependent prolongation of APD as a result of inhibition of the inwardly rectifying potassium current (I_{K1}) is abolished by the application of an opposing outward current, indicating this is an intrinsic property of the cardiac membrane and not explained by the mode of action of $BaCl_2$. Reproduced with permissions from Bányász *et al*.[4]

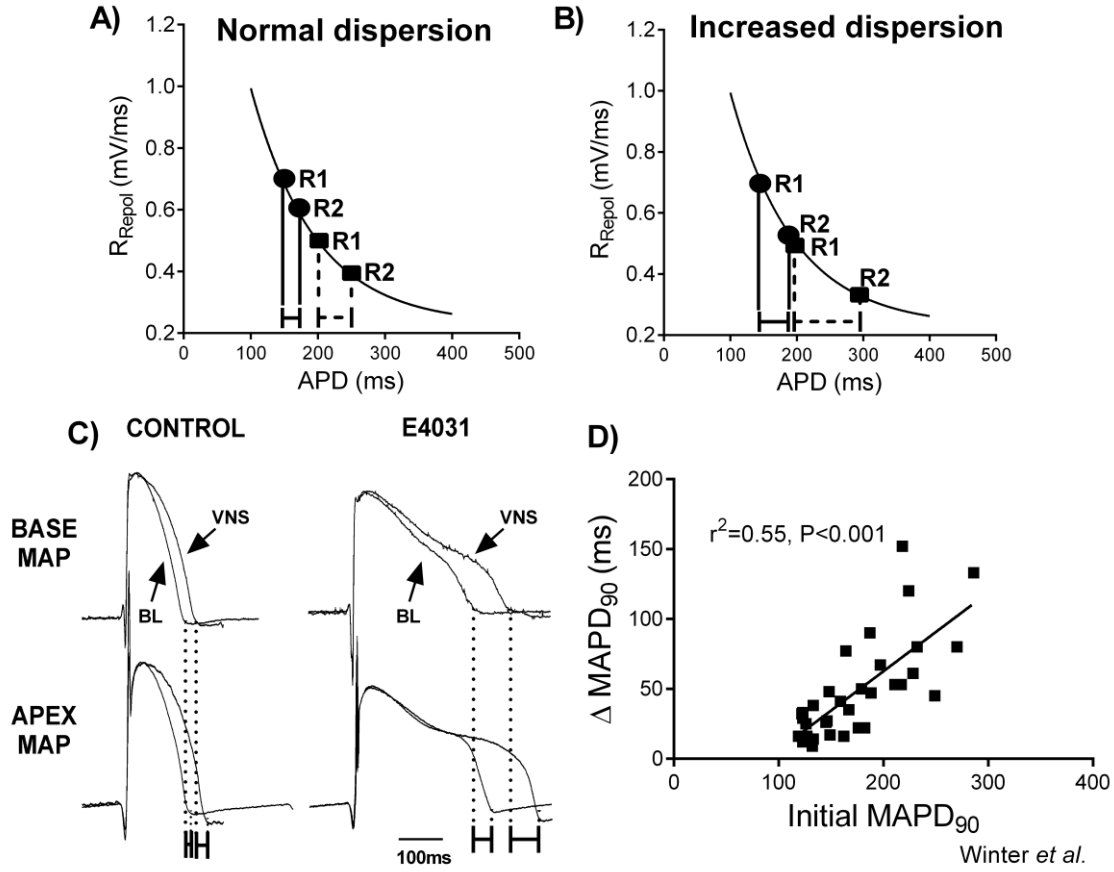

Figure 4. Rate dependence of dispersion of repolarization. A) Modeling the effect of heart rate on regional action potential duration (APD). Slowing of heart rate is represented as a uniform reduction (0.1 mV/ms) in the rate of repolarization (R_{Repol}) in two ventricular regions with different APD (R1 and R2). The initial difference in APD (dispersion) between the two regions is predicted to increase when heart rate is reduced from fast (circle) to slow (square) rates. B) The increase in dispersion with bradycardia is predicted to be dependent upon the starting conditions, whereby the effects of slowing rate are magnified when the initial difference in APD between the two regions (R1 and R2) is greater. C) Monophasic action potential recordings from an isolated rabbit heart demonstrating the increase in dispersion of repolarization (dotted lines) associated with vagally mediated bradycardia. Note the greater prolongation of action potential duration in apical regions, which are longer to begin with. Treatment with E4031, a selective inhibitor of the rapid delayed rectifying potassium current, increases baseline dispersion and potentiates the effects of bradycardia. D) Data from the same study demonstrating a correlation between initial monophasic action potential duration ($MAPD_{90}$) and the magnitude of change in $MAPD_{90}$ during bradycardia (n=8 hearts). Data reproduced with permissions from Winter *et al.*[35]

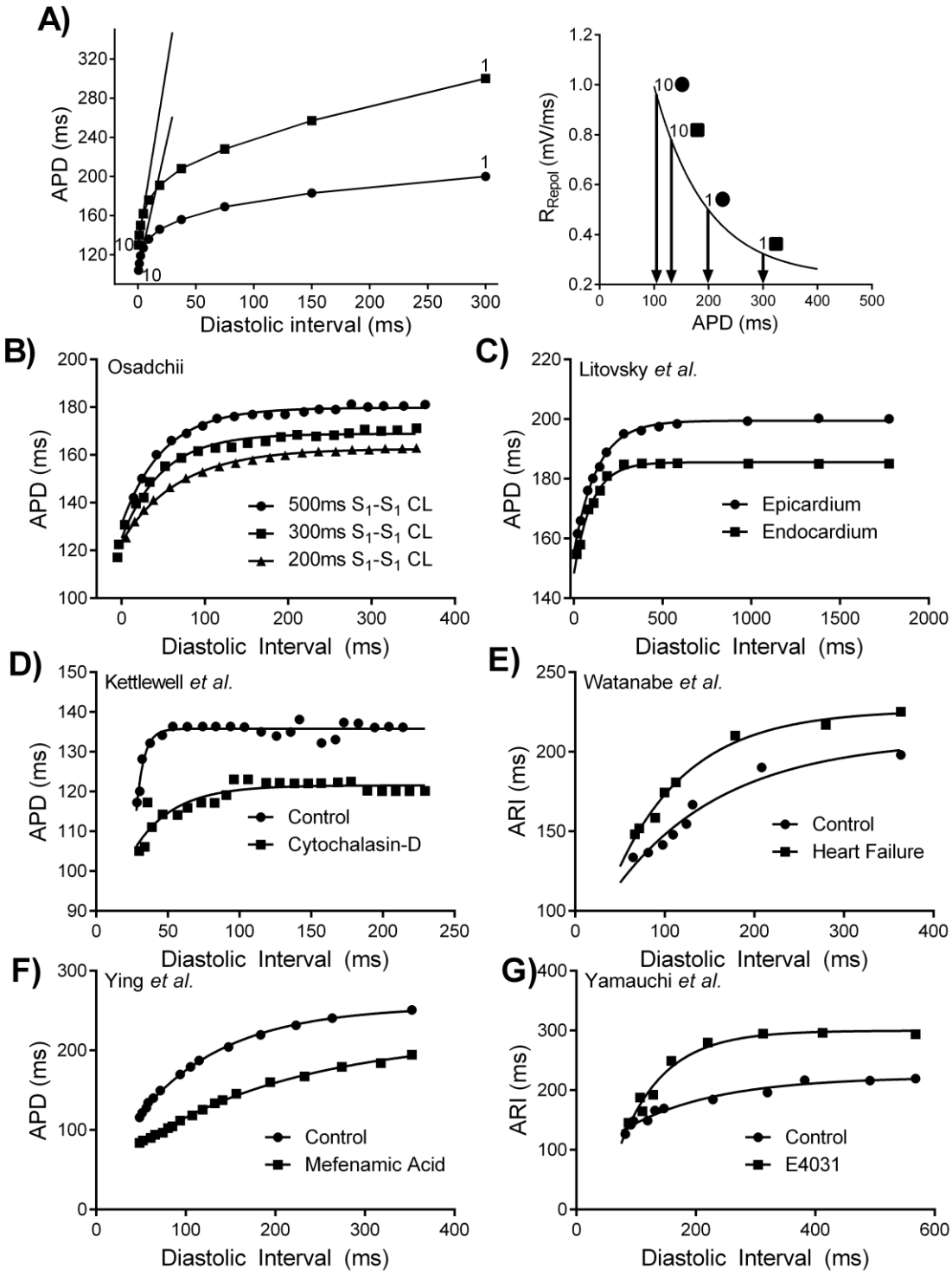

Figure 5. Action potential duration restitution kinetics. A) Predicted effect of an increase in action potential duration (APD) on the APD restitution (APD-RT) from the rate of repolarization (R_{Repol})-APD relationship. Curves were generated by plotting step increases in the R_{Repol} (0.025 mV/ms per step), from two different starting values for APD (denoted by the rightmost arrows (1)), for a total of 10 steps (final values denoted by the leftmost arrows (10)). This models the shortening of APD during restitution and predicts that a longer action potential will be associated with a greater magnitude of APD shortening over a given range of DIs, a steeper APD-RT slope and that curves will converge towards the same point. This prediction is supported by

experimental data assessing differences in APD-RT with B) different pacing rates (guinea-pig),[46] C) between endocardial and epicardial myoyctes (dog),[84] D) with the electromechanical uncoupling agents 2,3-Butanedione 2-monoxime (rabbit),[55] E) in pacing induced heart failure (dog),[42] and with drugs that F) augment (pig)[50] or G) block outward potassium currents (dog).[47] Data re-plotted for clarity, with permissions. ARI=activation recovery interval.

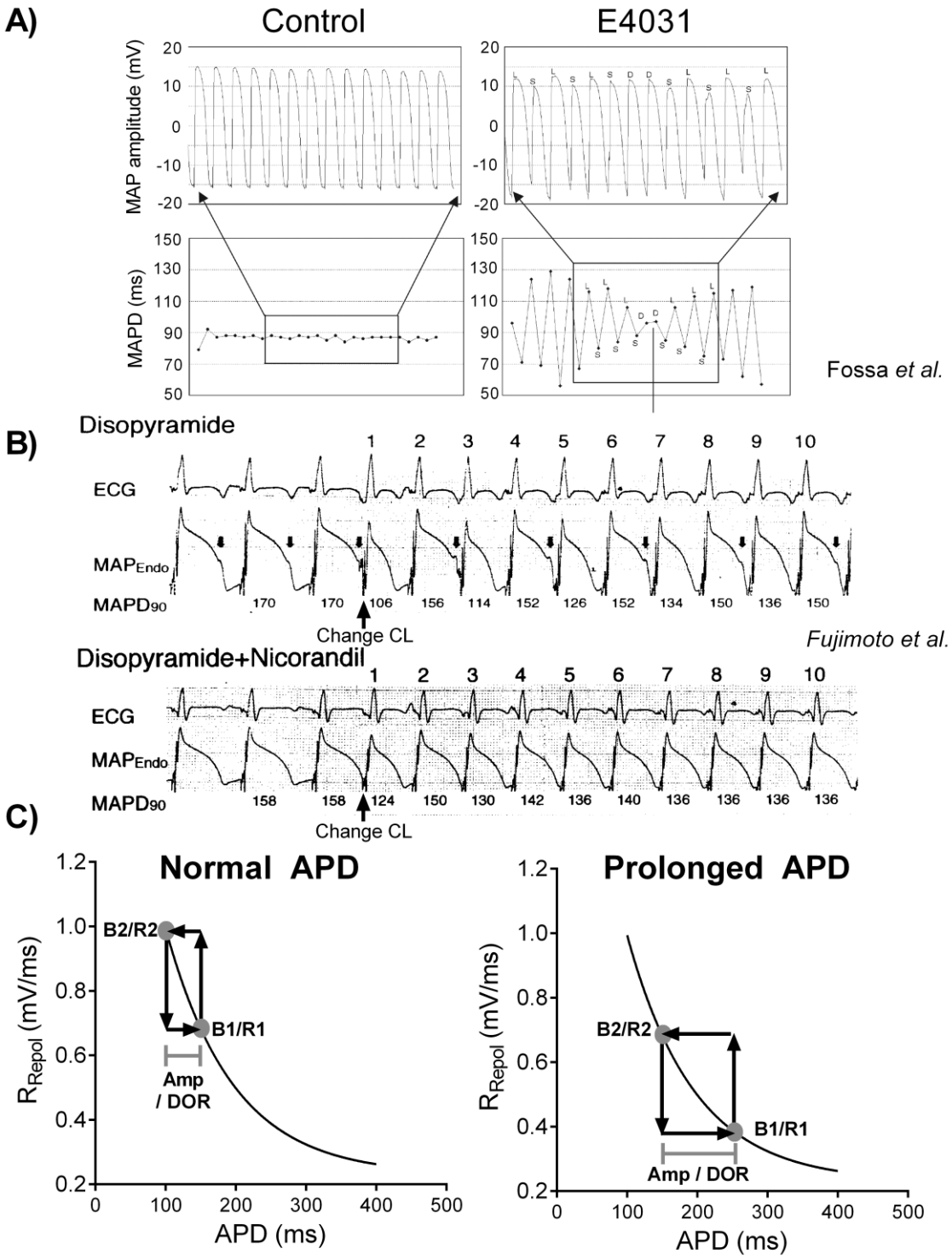

Figure 6. Electrical alternans. A) Experimental data from anaesthetized guinea pigs. Significant electrical alternans are observed during rapid pacing (150ms cycle length (CL)) when APD is prolonged by E4031 treatment (23 nmol/L), but not in control conditions. Data reproduced with permissions from Fossa *et al.*[65] B) Data from isolated guinea pig hearts demonstrating the effect of a sudden decrease in cycle length (CL) on the magnitude of electrical alternans in electrocardiogram (ECG) and monophasic action potential (MAP) recordings. Treatment with nicorandil shortens the action potential and reduces the amplitude of alternans associated with disopyramide treatment. Data reproduced with permission from

Fujimoto *et al.*[67] C) Predicted effect of beat-to-beat oscillations in the rate of repolarization (R_{Repol}) on action potential duration (APD), reflecting cycling of intracellular calcium transient amplitude at fast heart rates. Data are an example of positive calcium to voltage coupling. When APD is prolonged the same magnitude change in the R_{Repol} (0.3 mV/ms) is associated with a greater change in APD, as can be seen from the difference between beats 1 (B1) & 2 (B2). Amp = alternan amplitude. In the setting of spatially discordant alternans dispersion of repolarization (DOR), between two out of phase regions (R1 and R2), is predicted to be greater when the action potential is prolonged.